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Browning inhibition and microbial control in fresh-cut persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante) by apple pectin-based edible coatings

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ABSTRACT

The aim of this study was to develop new edible coatings based on apple pectin with a combination of antioxidants and antimicrobial agents to control enzymatic browning and microbial growth of fresh-cut 'Rojo Brillante' persimmon. The survival of important food-borne human pathogens artificially inoculated on fresh-cut fruit was also assessed. Potassium sorbate (PS) at 2 or 4 g kg^{-1}, sodium benzoate (SB) at 4 g kg^{-1}, or nisin (NI) at 500 IU mL^{-1}, were added to apple pectin coatings containing 10 g kg^{-1} citric acid and 10 g kg^{-1} calcium chloride as antioxidants. Persimmon slices were dipped in the coatings, the aqueous antioxidant solution (citric acid and calcium chloride) or water (control), packed in an ambient atmosphere and stored at 5 °C for up to 9 days. Microbial growth, colour, firmness, polyphenol oxidase (PPO) activity, visual quality and overall sensory flavour were measured during storage. Coated samples and those dipped in the antioxidant aqueous solution presented lower a^* values than control samples, which indicated effective browning inhibition. Persimmon slices treated with coatings containing PS and SB reached the limit of marketability after 7 days of storage. At the end of storage, the overall fruit flavour was ranked above the limit of acceptability. Antimicrobial coatings inhibited growth of mesophilic aerobic bacteria, and those containing SB and NI were the most effective. No growth of moulds, yeasts and psychrophilic aerobic bacteria was detected during storage. All the treatments effectively reduced the populations of *Escherichia coli* and *Salmonella enteritidis*, NI-coating being the most effective. For *Listeria monocytogenes*, only the NI-coating effectively reduced the bacterial population.

1. Introduction

'Rojo Brillante' persimmon is an important cultivar in the Ribera del Xúquer area (Valencia, Spain). When harvested, it is an astringent variety, but the application of high CO_2 levels allows the removal of astringency without affecting fruit firmness (Salvador et al., 2007), which enables this fruit to be commercialised as a fresh-cut commodity. However, fruit processing promotes faster deterioration due to tissue damage, which leads to increased physiological activity and major physico-chemical changes, such as enzymatic browning, softening, etc. During processing, spoilage and pathogenic microorganisms can also contaminate the product surface, and the nutrients inside the fruit contribute to their growth. Post-processing contamination or recontamination of the surface of food products by these pathogens has led to recalls and outbreaks of food-borne illness (Reij and Den Aantrekker, 2004). Although the growth of human pathogens on the flesh of fresh fruit is thought to be limited due to acidity, recent studies have documented the exponential growth of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on a variety of fresh-cut fruit (Alegre et al., 2010). Pathogen growth has also been demonstrated in non-acidic fruit, such as melon, watermelon, papaya and mango (Penteado and Leitao, 2004; Strawn and Danyluk, 2010).

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In recent years, the use of edible coatings has emerged as a new, effective, and environmental-friendly alternative mean to extend the shelf life of many products, including fresh-cut fruit and vegetables, by providing a barrier to water loss and gas exchange. Furthermore, their functional properties may be enhanced by the addition of food ingredients, such as antioxidants and antimicrobials, to enhance appearance, integrity and microbial safety, among others (Valencia-Chamorro et al., 2011b). The basic ingredients of edible coatings are proteins, polysaccharides and lipids; whereas, active ingredients include other generally regarded as safe compounds (GRAS) and food-grade additives to meet international regulations that considers edible coatings as part of the food (EU Directive 98/72/EC, 1998; US FDA, 2006). In previous research works by our group, a pectin-based edible coating containing 10 g kg^{-1} citric acid (CA) and 10 g kg^{-1} calcium chloride ($CaCl_2$) proved effective among different polysaccharide coatings to control the enzymatic browning of fresh-cut 'Rojo Brillante' persimmon (unpublished data). This effect was attributed to the capability of pectin to form strong insoluble polymers upon the reaction with multivalent metal cations like calcium (Oms-Oliu et al., 2008a).

Microbiological stability is also a critical factor to maintain the commercial marketability of fresh-cut produce. Incorporating antimicrobial compounds into edible coatings is becoming an important practice for the potential development of novel treatments for fresh-cut fruit as it helps to reduce the deleterious effects of processing. The use of these substances has its advantages over the direct application of antibacterial agents onto foods because edible films can be designed to slow down the diffusion of antimicrobials from food surfaces. The effectiveness of different antimicrobial substances, such as lysozyme, nisin (NI), organic acids, essential oils and their derivatives incorporated into edible films against several pathogens has proven satisfactory (Rojas-Graü et al., 2009; Valencia-Chamorro et al., 2011b). Among them, essential oils are the most studied antimicrobial ingredients incorporated into edible coatings against pathogenic microorganisms in fresh-cut fruit. However, in many cases effective concentrations adversely affected the sensory properties of coated fruit (Rojas-Graü et al., 2009; Valencia-Chamorro et al., 2011b). On the other hand, potassium sorbate (PS), sodium benzoate (SB) and NI are widely used by the food industry as safe antimicrobial food additives, although they have been less studied as edible coating ingredients to control microbial growth in fresh-cut fruit. Nevertheless, some studies have proved the antimicrobial activity of a cellulose-based edible coating amended with 1 g kg^{-1} SB or PS in fresh-cut apple and potato (Baldwin et al., 1996) and a starch-based coating containing 2 g L^{-1} PS on fresh strawberries under cold storage (García et al., 2001). A more recent work also reported that the application of cellulose films containing 7500 IU mL^{-1} NI inhibited the growth of *Staphylococcus aureus* and *L. monocytogenes* in processed mangoes (Teixeira-Barbosa et al., 2013). However, no research studies about the effect of incorporating these compounds into pectin-based edible coatings applied to fresh-cut persimmon to ensure quality and safety have been published. Therefore, the aim of this work was to determine the effects of different antimicrobial agents, incorporated into an optimised apple pectin-based edible coating, on fruit quality and microbial growth of fresh-cut 'Rojo Brillante' persimmon. The survival of important food-borne human pathogens artificially inoculated on fresh-cut fruit was also assessed.

2. Materials and methods

2.1. Plant material

Persimmons (*Diospyros kaki* Thunb cv Rojo Brillante) at commercial maturity were provided by a local packinghouse assigned to the persimmon geographical indication 'Denominación de Origen Kaki Ribera del Xuquer' (Valencia, Spain). Persimmons were harvested with an average external colour index ($CI = 1000 \times a/L \times b$) of 13.29 ± 3.17, firmness of 45.76 ± 6.69 N, total acidity of 38.17 ± 4.06 g of malic acid per 100 g and soluble solid content of 15.13 ± 0.31 °Bx. Before the experiments, fruit were selected for size and absence of physical damage and randomly divided into 6 groups, which corresponded to 4 coating treatments, 1 antioxidant-dipped treatment, and 1 water-dipped control. The persimmons were free of any postharvest treatment.

2.2. Edible coatings formulation

Edible coatings were elaborated from a base solution of apple pectin (Sigma–Aldrich, St. Louis, MO, USA) at 10 g kg^{-1}. Aqueous solutions of apple pectin were prepared at mild heating. Glycerol (Panreac Quimica, S.A., Barcelona, Spain) was added as a plasticizer at 10 g kg^{-1}, and coating solutions were emulsified with 2.5 g kg^{-1} oleic acid (Panreac Quimica, S.A.) and 2.5 g kg^{-1} Tween 80 (Sigma–Aldrich). As antioxidant agents, 10 g kg^{-1} citric acid (CA; E-330) (Quimivita, Barcelona, Spain) and 10 g kg^{-1} calcium chloride ($CaCl_2$; E-509) (Sigma–Aldrich) were incorporated into the coating formulations. The antimicrobial agents tested were potassium sorbate (PS; E-202) at 2 or 4 g kg^{-1}, sodium benzoate (SB; E-211) at 4 g kg^{-1}, or nisin (NI; E-234) at 500 IU mL^{-1}. All these ingredients are classified as food additives (with their correspondent E-number) or GRAS compounds by the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (US FDA) and the concentrations tested were within the legal limit. PS and SB were supplied by Sigma–Aldrich Chemie (Steinheim, Germany) and NI was acquired from Coralim Aditivos S.L. (Valencia, Spain). Coating solutions were kept at 5 °C until application.

2.3. Pathogenic strains and inoculum preparation

Stock cultures for the food-borne contamination-specific human pathogenic strains of *E. coli* serotype O157:H7 (CECT 4972; ATCC 700728), *Salmonella enterica* subsp. *enterica* (CECT 4300; ATCC 13076) and *L. monocytogenes* serovar 1 (CECT 7467; ATCC 19111) were obtained from the Microbiology Reference Laboratory (University of Valencia, Spain) in the form of agar slants. Strains were activated by streaking on MacConkey's agar (AES Laboratoire, Combourg, France) (*E. coli* and *Salmonella enteritidis*) and tryptic soya agar + 50 g kg^{-1} sheep's blood agar (BD, New Jersey, USA) (*L. monocytogenes*) plates, followed by incubation for 48 h at 37 °C. Single colonies were grown individually in Luria–Bertani broth (Luria–Bertani®, Barcelona, Spain) (*E. coli* and *S. enteritidis*) or tryptone soya yeast extract broth (Sigma–Aldrich Chemical Co., St. Louis, MA, USA) (*L. monocytogenes*) for 24 h at 37 °C. Bacterial cells were harvested by centrifugation at 3,000 rpm for 10 min at 10 °C and then resuspended in saline peptone to obtain a concentrated suspension. The process was repeated 3 times. Finally, cell pellets were resuspended in maximum recovery diluent to obtain a culture optical density of 0.2 at 600 nm. This corresponded to a final inoculum concentration of 6.0 log cfu mL^{-1}.

2.4. Persimmon processing and packaging

Natural astringency of 'Rojo Brillante' persimmons was eliminated by placing them for 24 h in closed chambers at 20 °C with an atmosphere containing 95 ± 2 kPa CO_2. Chambers used for deastringency consisted of hermetically sealed, transparent polymethyl methacrylate cabinets ($82 \times 62 \times 87$ cm) fitted with outlet and inlet ports through which CO_2 (Alphagaz, Air Liquide